Characterization of Different Components and Selective Improvisation of Beers

कैरेक्टराइजेशन ऑफ़ डिफरेंट कंपोनेंट्स एंड सेलेक्टिव इम्प्रोवाइजेशन ऑफ़ बीयर्स

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by

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Detailed synopsis for registration to Ph.D. program in Food Science and Technology

Proposed title of Ph.D. thesis: Characterization of Different Components and Selective Improvisation of Beers

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INTRODUCTION

Beer is the most consumed alcoholic beverage worldwide (Colen & Swinnen, 2011). Beer is obtained after yeast alcoholic fermentation of brewer's wort, which is composed of barley malt, water and hops. The different combinations of ingredients and brewing processes yield a chemically complex product, which present numerous types and styles. Beers are primarily classified according to the fermentation process as top or high, and bottom or low fermentation beers (Wunderlich & Back, 2009).

Lagers, the most consumed type of beer, are produced by "low" fermentation, which is carried out under refrigeration (usually between 6ºC and 15ºC). After fermentation, yeast cells deposit at the bottom of the fermentor and are usually removed. In contrast, ale type beers are produced by "high" fermentation, occurring between 16ºC and 24ºC, after which yeast cells rise to the surface of fermentation media, forming a thick film that is generally not completely removed.

Different beer styles, such as pilsner (standard American lager), bock, weizen, pale and brown ales, rauchbier and many others originate from variations in processing, formulations and ingredients composition. Additional classification of beers are based on changes in brewing processes, such as for production of draft beers, which are non-pasteurized, and non-alcoholic beers, often produced by limited fermentation. Beer styles may also vary among producing regions, according to cultural aspects and ingredients availability (Bamforth, 2003).

Brewing

The production of beer is called brewing. Each ingredient has its own function.

Barley provides the starch which is converted to maltose and other sugars, and finally to alcohol and carbon dioxide. Color, flavor and body are other important functions of barley and are dependent on its roasting method. Different degrees of roasting result in a range of colored beers from very light to very dark.

The major steps in producing malt and beer include: barley intake, cleaning and grading, drying, steeping, germination and kilning malt, roasting, milling, mashing, boiling, fermenting, maturing, filtration and packaging or bottling.

Barley is one of the founder crops of old world agriculture and is one of the first domesticated cereals. It is fourth largest cereal crop after wheat, rice and maize It is a major source of food for large population of cool and semi-arid areas of the world, where wheat and other cereals are less adapted. In the world barley have the share of 7% of the global cereal production.

Malt is the product coming from processed barley that has been steeped in water, germinated on malting floors or in germination boxes or drums, and later dried in kilns, under carefully controlled conditions. Malt is used predominantly as the basic raw material for beer and spirit, with a much smaller quantity used in the food industry.

Malt is milled (ground) and mixed with water. During the subsequent procedure, called mashing, high molecular mass components (starch, polymerized carbohydrates) of malt are degraded by enzymes at specific temperatures.

The suspension obtained is then filtered to separate the liquid (wort) from the spent grains. This process is called lautering.

The wort is subsequently boiled with the addition of hops; the high temperatures cause a coagulation of constituents, which are then called hot trub. They are separated together with the solids from the hops (hot trub removal). The clarified wort is subsequently cooled to the pitching temperature required by the fermentation method and the yeast strain used.

The fermentation process is initiated by pitching: this consists of saturating the cold wort with air, and adding cultured yeast. The fermentable low molecular mass components of the wort (glucose, maltose, etc.) are converted to ethanol and numerous aroma compounds according to the metabolism of the yeast. After maturing the taste and enriching with carbon dioxide produced by the fermentation, the beer is filtered to clearness, and bottled. After bottling pasteurization process (heat the beer at 63ºC and immediately cooled at 50ºC or below) was completed.

Antioxidants are "any substance that when present at low concentrations compared with those of an oxidizable substrate significantly delays or prevents oxidation of that substrate". Antioxidants act in various ways, which include complexation of redox-catalytic metal ions, scavenging of free radicals, and decomposition of peroxides. The intensity of these effects depends on the chemical structure and concentration of the AO present. Natural antioxidants may also protect DNA, protein, and membrane lipids from oxidative damage in biological systems, and provide additional health benefits for disease prevention and health promotion (Tafulo et al., 2010).

In the production process, the addition of hops, cereals and malt leads to an increased content of naturally occurring antioxidant compounds in beer, mainly phenolic compounds, and also Maillard reaction products, and sulfites. The structural classes of polyphenols in beer include simple phenols, benzoic and cinnamic acids derivatives, coumarins, catechins, di- and tri-oligomeric proanthocyanidins, prenylated chalcones and α- and iso-α acids. It is worth mentioning that phenolic compounds influence beer flavor and are associated with beer chemical stability and shelf life enhancement. Because of the influence on beer sensory quality and stability, there is interest in determining phenolic compounds contents across beer types and styles.

REVIEW OF LITERATURE

Malt is produced from barley grains in a process called malting and is used in beer production as a source of starch, contributing to beer's color and organoleptic characteristics. Malt also plays an important role in the oxidative stability of beer and as a natural source of antioxidants that can limit reactions caused by reactive oxidizing species (ROS). Antioxidants from malt are able to scavenge oxygen-free radicals and prevent oxidative reactions, avoiding the addition of exogenous antioxidant compounds that could be used to maintain the oxidative stability of beer. Moreover, malt antioxidants may bring important benefits to a consumer's health, preventing and neutralizing ROS known to be associated with numerous diseases.

The malting process and roasting are responsible for changes in the composition of barley and malt grains, involving modifications and degradation of endogenous phenolic compounds and the generation of Maillard reaction products (MRPs) with a great impact on the overall

antioxidant capacity of malt. In fact, the development of MRPs during thermal processing has been associated with a pro-oxidative effect and with a negative effect on the oxidative stability of malt and beer. The mechanisms behind anti/prooxidant effects of MRPs are still unclear since their structures are still unknown. The mechanisms are assumed to be based on their ability to trap positively charged electrophilic species, to scavenge oxygen radicals, to have reducing power, and to chelate metals to form inactive complexes.

Wheat, one of the oldest cultivated crops that has been grown as food at least since 8000–10,000 BC (Pomeranz, 1998), and has a long tradition as a raw material for the production of malt and beer. Nevertheless, it has been studied to a much lesser extent than barley. Wheat beer is a specialty beer that evolved in Bavaria in the late Middle Ages. Historically, the key difference between what was then labelled 'brown beer' and 'white beer,' was the type of fermentation – a distinction that has since been included in the modern version of the German Beer Purity Law (Vorläufiges Biergesetz). According to that prescription, all wheat beer brewed in Germany must be top-fermented, made with at least 50% malted wheat, and have an original gravity of at least 11°P. For optimal brewhouse results, within the confines of this law, efforts have been made in recent years to breed and cultivate wheat varieties that are suitable for wheat beer-making. Before the feudal purity law (Reinheitsgebot) took effect in 1516, brewers used just about any available raw material for fermentation and flavouring. Mashes consisted of barley, or wheat, and/or oats and the brews were flavoured with ingredients such as herbs, willow bark, even oxen bile. However, such unusual ingredients often resulted in completely undrinkable concoctions. As far as wheat beer is concerned, today's feudal purity law, Germany's 'provisional beer law,' has evolved from that very description. It now stipulates, and it is also stated in the World Beer Cup Style Guidelines, that 'white beer' must be made from at least 50% malted wheat and be top-fermented. Also an original gravity (ºP) 1.047–1.056 (11.8–14 ºP), apparent extract/final gravity (ºP) 1.008–1.016 (2–4 ºP), alcohol by weight (volume) 3.9–4.4% (4.9–5.5%), bitterness 10–15 IBU and color of SRM (EBC) 3–9 (6–18 EBC) comprises a south German-style Hefeweizen. It must also have a 'typical' flavor and aroma. Throughout its long history, the popularity of wheat beer has fluctuated greatly. German wheat beer consumption was still confined almost entirely to the southern regions of the country. However, in recent years that situation has changed, with Weissbier production almost doubling between 1990 and 2009, in spite of a concomitant overall decline in German beer consumption. In other

countries, wheat beers are also gaining market share. This includes the classic Belgian wheat beer style called ‘wit’ in Flemish and ‘bière Blanche’ in French. Belgian wheat beers tend to be brewed with plenty of unmalted wheat. They may also exhibit slightly sour and spicy notes from the addition of coriander and bitter orange peel.

As the production of wheat beer increases, so does the demand for suitable brewing wheat. While breeding and cultivation efforts for barley have been highly successful in creating agronomically and brew-technical optimal specialty cultivars that have become well established as brewing barley varieties, the picture is completely different for brewing wheat, even though wheat is the world’s second-most planted grain crop after corn. An increasing wheat beer yield results in a rising amount of raw material. It is this high demand that defines most of the wheat breeding objectives, and these objectives are generally not favourable to brewers. Baking wheat should be as high in protein as possible, and it should have stable Hagberg Falling Numbers (high numbers indicate low levels of enzyme activity and vice versa). A high protein content, which may be a boon for farmers and bakers, is a bane for brewers, since it can cause long lautering times, filtration difficulties and fermentation problems in the brewery, as well as decreased flavor stability in the finished beer. Thus, wheat in brewing has been somewhat overlooked from a scientific perspective.

Wheat beer shows a big variation in its production and brewing process. Beer flavor occurs owing to the interactions of diverse flavor compounds such as alcohols, esters, carbonyl compounds, organic acids, phenolic substances and sulphuric compounds. Back et al. (1998) attempted to categorize wheat beers in terms of their dominant flavor orientations. He divided them into four main groups: (a) ester-like; (b) phenolic-like; (c) neutral; and (d) yeast type. Esters have an enormous influence on the flavor of wheat beers. Many wheat beers also have a strong ‘malty’ component. With the exception of the ‘neutral’ type, wheat beers tend to be much more aromatic than bottom-fermented brews. Chemically, the characteristic phenolic, clove-like flavour stems from4-vinylguaiacol (4-VG), the fruity, aromatic, banana-like flavor from isoamyl acetate, and the estery component from ethyl acetate. The sensorial threshold is very low and can be characterized as aromatic in marginal concentrations. Furthermore, wheat beer can also have a typically ‘malty’ component (maltol, furaneol).

Wheat beer has more unique aromatic compounds compared with lager beer. Essential for the phenolic aroma is 4-VG, and for the estery flavour isoamyl acetate and ethyl acetate, whereas middle chain fatty acids are responsible for off-flavours. The influence of malt on the aroma compounds depends primarily on the malt technology. A correlation between the aroma relevant esters (3-methylbutyl acetate and 2-methylbutyl acetate) and the content of the corresponding amino acids, such as leucine and isoleucine, has been found. For the formation of sufficient 4-VG, the amount of ferulic acid in the sweet wort is essential. The author also reported that no differences in aroma compounds were found between decoction and infusion processes (Faltermaier et al., 2013).

Dark specialty malts can be defined as all malts cured at higher temperatures than pilsner malt or roasted in a roasting drum. Depending on the production process, these malts are usually classified into three groups: color malts, caramel malts and roasted malts (Coghe et al., 2003). The most important properties of dark malts (color, flavor and antioxidant activity) result from non-enzymatic browning, also known as the Maillard reaction. Color malts, but also caramel malts and roasted malts, which are rarely used in levels above 5%, can have an appreciably high impact.

On the final beer characteristics as they contribute to the color and flavor of several beer styles. An unlimited number of colors and flavors can be attained by the use of different dark specialty malts, but also by the variation of the dark malt level or by the combination of different malt types. Dark malts may also improve the foam stability and the mouth feel of beer, presumably by the presence of melanoidins. Furthermore, due to a higher level of antioxidants, beers brewed with dark malts normally have a better flavor stability and a longer shelf life than pale beers. The influence of non-enzymatic browning on both physicochemical as well as sensory characteristics of malt was assessed in previous work. Conversely, this study was mainly concerned with the effect of dark malts and, as a consequence, of non-enzymatic browning on the course of the fermentation and on the development of flavor active yeast metabolites. During fermentation, brewing yeast strains utilize a wide variety of nutrients to support growth and to generate energy. Of these, carbohydrates and amino acids are of most significance for fermentation performance and beer quality. As, during thermal treatment of malt, Maillard reactions are initiated by the reaction between reducing sugars and amino compounds, it can be

deduced that wort brewed with considerable levels of dark malt contains lower levels of carbohydrates and amino acids. This undoubtedly affects several aspects of the fermentation process, including attenuation and fermentation rate. Since the amino acid metabolism of brewers' yeast is closely associated with the formation of fusel alcohols, esters and vicinal diketones, lower amino acid levels in wort brewed with dark malt might substantially affect the generation of these important flavor-active compounds. To gain more knowledge on the levels of available yeast nutrients, fermentable carbohydrates and amino acids were quantified in Congress wort. Furthermore, pilot scale wort samples were brewed with different levels and different types of dark specialty malts and assessed for fermentation rate and attenuation, while flavor compounds were evaluated in the resulting beers. In order to better detect the impact of Maillard compounds, brews were prepared with excessive levels of dark malt and without the addition of hops (Coghe et al., 2005).

Hop, which is added during the boil, is the source of bitter compounds and responsible for the bitter taste in beer. The hop's antioxidant activity depends on the hop variety, quality and method of processing (Lermusieau et al., 2001). The total content of polyphenols in hops usually ranges between 2 and 6% depending on the hop cultivar (Liu et al., 2007). Most of the dried hops are processed into the hop products, while only a small percentage (2%) are marketed in a form of raw hops. Typical hop products are hop pellets (60%) and extracts (20%), while the rest is allotted to isomerized products (Biendl, 2010). The amount of phenolics and their antioxidant activities may vary widely depending on the postharvest processing and applied extraction conditions. The highest total phenolic and flavonoid concentrations in hop extracts (Magnum and Marynka varieties) and their pellets (T90 and T45) could be achieved by extraction with 50% ethanol (Kowalczyk et al., 2013). The α-acids do not contribute significantly to the reducing power of hop. Hop extracts with a high level of bitter substances and almost no polyphenols display the least ability to confer reducing power to the boiling wort. However, the antioxidant activity is due to the α-acid's existence. The Saaz variety is well known for its good flavor and as a very stable variety regarding the changes in polyphenols. It is also well known that polyphenols, especially hop polyphenols, slow down flavour deterioration in beer during storage (Mikyska & Krofta, 2012).

Various adjuncts like **fruits** and **spices** might also be used to contribute to the flavor and aroma profile of some beer styles. There are several traditional beer styles which are brewed with spices, for example, coriander is used in Belgian Witbier and Finnish Sahti is brewed with juniper berries. Similarly, different fruits such as cherry, raspberry, and peach have been used as a beer adjunct or as a flavoring agent for centuries. Fresh fruits such as cherries and raspberries are mixed to the finished beer and allowed a further fermentation to prepare a special fruit beer in Belgium. The fruits used in beer preparation are not essentially fermented; they might be rather added only for imparting the fruit flavor to the finished beer. Among the various starch-containing beer adjuncts, the addition of fruit is common to wheat which also contributes to a good texture. A Belgian fruit beer, Fruli is prepared from 70% wheat beer and 30% strawberry fruit juice. (Cho et al., 2018)

The DPPH radical-scavenging activity of beer samples increased with the amount of persimmon added. Similarly, the superoxide anion scavenging potential of beer samples significantly increased with the addition of persimmon fruit. The effect of persimmon fruit was also reflected in the hydroxyl radical scavenging potential of the beer samples as in the DPPH and superoxide anion. the persimmon-added beer with good scavenging capacity could be one of the potential protective sources against harmful free radicals.

Total polyphenol content of the beer samples significantly increased with the amount of the fruit added. The high total polyphenol value for the persimmon added beer samples was due to the large amount of phenolic compounds present in the persimmon fruits. The phenolic compounds polyphenols, flavonoids, and flavonols account for the antioxidant potentials of beer.

The pH and Titrable acidity were significantly increased but alcohol concentration remained unaffected. The beer samples prepared with persimmon fruit showed significantly high antioxidant potentials measured through DPPH and superoxide and hydroxyl anions scavenging potentials as well as total polyphenol contents compared to the control. The amount of mineral elements like Mg, K, and Ca was also significantly increased in the persimmon beer. Overall acceptance value of the persimmon beer prepared with the addition of 150 g of persimmon fruit in 10 L of water was the highest among the beer samples. The results of the present study showed that addition of 150 g of persimmon fruit per 10 L of water could better enhance the nutritional, organoleptic, and antioxidant potential of beer. (Cho et al., 2018)

Generally, the **mashing** process results in significant increases in phenolic content, and since mashing takes place under conditions suitable for enzyme action, it is likely to lead to the release of bound phenolics. Indeed, all monitored phenolic compounds including catechin, ferulic, vanillic, chlorogenic, and *p*-coumaric acids were significantly increased during mashing (Pascoe et al., 2003). Zhao and Zhao (2012) examined the evolution of phenolic content during mashing and presented that the TPC increased during the early stage of mashing and then decreased during the mashing at higher temperature, and the whole mashing process resulted in a 6.0% of increase in TPC. Other studies regarding the evolution of free and bound ferulic acid during the wort production process performed by Vanbeneden et al., (2008) and Szwajgier et al., (2005) also indicated that the ferulic acid concentration increased continuously from the start of mashing-in until the heating of the wort at 62°C or 75°C. And the highest amount of ferulic acid was released during the first 15 min of mashing and no additional ferulic acid was released during the subsequent operations. However, Szwajgier (2009) observed a decrease in the phenolic acid content from the very beginning of the mashing process. There was a controversy regarding the effects of boiling on the phenolic contents. For example, Vanbeneden et al., (2008) found that boiling the wort with hops caused a slight decrease in bound ferulic acid concentration, while no significant influence in free ferulic acid content was observed; whereas Szwajgier et al., (2005) reported a net increase in the ferulic acid content during wort boiling.

OBJECTIVES

- Physicochemical analysis of barley malts, other ingredients and beers.
- Antioxidant properties, total phenolic contents of barley wort and other ingredients.
- Characterization of phenolic contents and antioxidant activity of different beers.
- Effects of processing on the phenolic contents and antioxidant activity of beer.

MATERIAL AND METHODS

- Beer production: Different types of beer will be brewed by using barley malts and different ingredients using standard brewing technique.
- Sensory evaluation: Sensory evaluation of beers will be done by selective panel.
- Physiochemical analysis: Various physiochemical analysis will be determined by suitable methods.
- Total phenolic contents: Total phenolic contents will be determined by suitable methods.
- Free radical scavenging capacity: Free radical scavenging capacity will be determined by suitable methods.
- Reducing power: Reducing power will be determined by suitable methods.
- Statistical analysis: Data obtained through experimentation will be statistically analyzed by using suitable method.

SIGNIFICANCE

Beer is one of the most commonly consumed alcoholic beverages with beneficial effects on human health. This is mainly true when “medium-strength beer” is ingested in a moderate level (light-to-medium consumption). Health advantages of beer (nutritional and medicinal) are associated with the presence of components, such as antioxidants, certain minerals, certain vitamins, fiber, as well as relatively low levels of ethanol.

Health related benefits

- Reduction of cardiovascular disease (cardioprotective effect)
- Anti-mutagenic and anti-carcinogenic effects
- Immune system stimulation (immunomodulation)
- Anti-osteoporosis effect
- Reducing risk of dementia
- Nutritional Benefits

All these benefits can be enhanced with the change in ingredients.

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